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Reduction of Enteric Methane Emission in a Commercial Dairy Farm by a Novel Feed Supplement

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Abstract

Several *in vitro* fermentation studies had demonstrated the efficacy of Mootral, a patent-pending natural feed supplement, in reducing methane gas production. In order to test the supplement's application in a commercial farm, Jersey (n = 121) and Holstein-Friesian (HF, n = 275) lactating cows received 500 g of pellets containing 3% Mootral powder for 12 weeks. Their enteric methane gas production was monitored using a hand-held laser methane detector. During 7 weeks of pre-Mootral supplementation period, dry matter intake (DMI), milk yield and milk quality (fat, protein, urea, pH, bulk tank somatic cell count (BTSCC)) were monitored for both herds and continued during 12 weeks of Mootral supplementation (Mootral period), and 4 weeks after withdrawal of Mootral (post-Mootral period). Feed samples were analysed during each period, and feed efficiencies (FE) for each herd were calculated. Compared to the baseline, the methane gas produced by the Jersey and HF cows during the Mootral period were lower by 38.3% and 20.7% ($p < 0.05$), respectively. DMI for Jerseys were greatest during the pre-Mootral period ($p < 0.05$), while no difference was recorded for the HF cows. Milk yield increased significantly ($p < 0.05$) for both herds during Mootral supplementation. FE increased significantly for the Jersey herd during the Mootral and post-Mootral periods ($p < 0.05$). Statistically significant changes were detected for urea in both herds, and BTSCC for HF cows, but these did not adversely affect milk quality. Results demonstrated Mootral reduces enteric methane production in lactating cows while increasing milk yield without affecting milk quality.

Keywords

Rumen Fermentation, Enteric Methane Mitigation, Milk Yield, Laser

Methane Detector, Dairy Farm

1. Introduction

A recent report by The International Panel on Climate Change has highlighted an urgent need to reduce greenhouse gas (GHG) emissions down to zero by year 2050 in order to limit the global warming rise of 1.5˚C [1]. The GHG emissions contribution from the global livestock sector was estimated at 14.5% of the global total, and two-thirds of this was from cattle, making it the largest contributor in this sector [2]. Furthermore, enteric emissions of methane accounted for 39.1% of GHG emissions from livestock supply chains [2]. This substantial GHG emission from cattle warrants mitigation efforts in order to reduce global warming.

Modulation of rumen fermentation had been shown to reduce enteric methane production [3] [4]. The methanogens, a subgroup of Archaea, are the known producer of methane gas during rumen fermentation [5], and various works have attempted to mediate the methane release either via feeding of different substrates, or by direct influence on the microorganisms themselves [6] [7].

The measurement of methane emissions as a proxy for the efficiency of the rumen have been undertaken in previous studies through the use of respiration chambers. This has involved one cow being monitored for a 24-hour period after acclimitisation of the animal to the chamber. Although the results provide detailed results only one animal is monitored at any one time. Recently a laser methane detector (LMD) has been used to measure enteric methane emissions from the cows. The LMD is a hand-held, portable diode laser absorption spectrometer. The LMD readings have been shown to correspond to measurements taken with a respiration chamber [8] and have been shown to be able to measure methane from a larger number of animals than other methods [9] [10].

Mootral (Mootral SA, Rolle, Switzerland), a patent-pending compound, containing garlic powder and bitter orange (*Citrus aurantium*) extracts, has been demonstrated to reduce methane production [11]. Eger *et al.* [12] showed that Mootral reduced the percentage of methanogens during *in vitro* fermentation and this could be linked to an observed reduction of enteric methane release and production rate. However, it is important to understand if these results translate to commercial farm conditions. Therefore, the aim of this study was to determine the effects of Mootral supplementation on enteric methane released by lactating dairy cows on a commercial farm. Effects of the supplementation on feed intake and milk production was also considered.

2. Materials and Methods

2.1. Animals and Experimental Diets

This study was conducted at a commercial dairy farm (Brades Farm, Lancaster, United Kingdom) with 396 dairy cows. The lactating Jersey (n = 121) and Holstein-Friesian (HF) cows (n = 275) were housed in separate barns with sand-bedded freestalls. Animals were handled in accordance with the Scotland's Rural College's (SRUC) ethical committee guidance on animal use for research.

A basal diet that consisted of a TMR (55% grass silage, 20% caustic wheat, 6% whey permeate; DM basis), supplemented with 19% DM of a milker's supplement (59.5% rapeseed meal, 14% protected rape meal, 12% maize distillers, 10% soya, 4% palm kernel expeller, 0.5% magnesium oxide; DM basis) was prepared daily in a mixing wagon (Powermix Pro; Shelbourne Reynolds, Bury St Edmunds, United Kingdom) and placed in feeding troughs in both barns. Prior to mixing, feeds were stored separately in silos and both herds received the same basal diet.

Both herds received Mootral (Mootral SA, Rolle, Switzerland) incorporated in pellets, formulated by a feed mill (Dugdale Nutrition, Clitheroe, United Kingdom) containing 3% Mootral, based on previous internal research, along with other feed (58% rape meal, 14% protected rape meal, 12% maize distillers, 9% Hipro soya, 3.5% soya hulls, and 0.5% magnesium oxide; DM basis). The pellets were delivered in 25 kg bags and stored in a dry, sheltered area in the farm. The pellets were supplemented at 500 g/day/cow in the TMR during the 12 weeks of Mootral supplementation (**Table 1**).

2.2. Monitoring

All cows on the farm were milked twice a day starting at 06:15 hours and again at 18:00 hours, in a swing-over milking parlour (GEA-Westfalia, Düsseldorf, Germany). Weekly milk yield was recorded with the milk management system (GEA-Westfalia, Düsseldorf, Germany). Milk from the Jersey and Holstein-Friesian herds were stored in separate cooled bulk tanks. Milk was sampled from separate bulk tanks for each herd every week in duplicate, for milk quality analysis. Samples were sent on the same day of milking to the National Milk Laboratories (NML; Wolverhampton, United Kingdom) for analysis of milk fat, protein, lactose, urea, pH and bulk tank somatic cell counts (BTSCC), using Fourier transform infrared spectroscopy (FTIR) (Foss, Hilleroed, Denmark). Redundancy testing for the same milk attributes was made with duplicate milk samples tested at Landeskontrollverband Weser-Ems (Leer, Germany) and the results from both laboratories were evaluated. As both laboratories showed comparable results, only the results from NML have been reported here. Milk yield and results of the milk analyses during Mootral supplementation were compared with the previous farm records.

Feed samples of the total mixed ration were collected in each period and sent for nutritional analysis (**Table 4**) using near infrared spectroscopy (Eurofins Agro, Wageningen, Netherlands). Dry matter intake (DMI) for the TMR fed to both herds was determined weekly from feed samples using a moisture tester (Koster, Ohio, USA) and the amount of refused feed was subtracted from the total initially provided.

Table 1. Schedule of sampling. FA = feed analysis, LM = laser measurement. FA of feed samples made at beginning of each period. The same schedule was applied to both Jersey and HF herds.

Week	−1	to	−7	1	4	8	12	13	to	16
Period	Pre-Mootral			Mootral				Post-Mootral		
	Milk yield records, weekly									
	Milk quality analysis, weekly									
							LM			LM
	FA			FA				FA		

Enteric methane emission from a sub-set of each herd were measured using a handheld LMD (Laser Methane Mini; Tokyo Gas Engineering, Tokyo, Japan). Measurements were taken individually from 15 Jersey and 15 Holstein-Friesian cows as they returned from the milking parlour in the morning from approximately 06:15 hours. Measurements procedure was similar to the work of Sorg *et al.* [13]. The cows were held in either a crush or artificial insemination stall and the handheld LMD was pointed at the cow's nostrils for 4 minutes at a distance of 1 m. LMD measurements were taken on Week 12 of the Mootral period with baseline measurements taken on week 16 (**Table 1**) from the same cows as identified by their unique ear tag numbers. Means of the LMD readings from each cow from these different periods were used for comparisons.

A qualified veterinarian observed the herds' health bi-weekly, taking note of any problems related with fertility, feet problems, mastitis, and metabolic disorders.

2.3. Calculations and Statistical Analyses

Feed efficiency (FE; L/kg) was the ratio of milk yield (L/day) to DMI (kg/day).

Weekly means of all variables of interest were analyzed using one-way ANOVA with post-hoc test on GraphPad Prism version 6.04 (GraphPad Software, La Jolla California, USA). The fixed effect was the period, while random effects were the LMD readings, DMI, milk yield; and milk fat, protein, lactose, urea, pH and BTSCC. Analyses were considered significant at $p \leq 0.05$.

3. Results and Discussion

3.1. Enteric Methane Emissions

Methane concentrations for both Jersey and HF cows measured with the LMD showed a significant decrease from the baseline values ($p < 0.05$) by 38.3% for the Jersey cows and 20.7% for the HF cows (**Figure 1**) with the use of the Mootral supplement. Data from 1 cow in the HF herd was omitted from analysis due to missing measurements in week 12.

Methane gas readings from the LMD in this study was low compared to other reported LMD measurements which ranged from 100 to 400 ppm-m [8] [13] [14]. Variations of methane emissions were influenced by the cow's activity, as demonstrated in an earlier work [14], and would explain the low methane

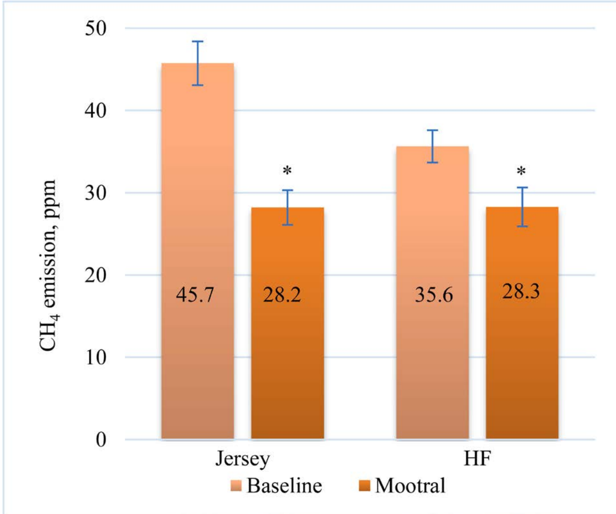

Figure 1. Mean enteric methane (CH_4) emissions for Jersey (n = 15) and HF (n = 14) cows during Mootral supplementation and baseline (* indicates significant difference), $p < 0.05$. Vertical bars are SEM.

measurements. As the LMD measurements were taken in the early morning before the cows had been fed, and with low rumen fill, fermentation would not have been at its peak. Measurements made in enclosed areas later in the day after rumen fill, as with respiration chambers, tended to record higher methane concentrations, as seen in LMD readings reported in an earlier work [8]. However, the LMD measurements were taken in a sheltered area with no noticeable effect of wind movements. When compared with other methods of methane measurements, the LMD still ranked intensity of methane similarly [13].

The results of enteric methane reduction previously reported for garlic additions in diets for ruminants have ranged from 6% to 18% [15] [16] [17], while that for citrus pulp had no effect on methane mitigation [18]. The bitter orange extract in Mootral would naturally contain flavonoids and as Graz and Miller [11] had shown, flavonoids alone did very little in reducing methane gas during rumen fermentation. However, when allicin from garlic was present with the bitter orange extract, there was notable methane reduction. This indicates that the Mootral composition could have synergistic effects in reducing enteric methane production.

3.2. Milk Yield and Milk Composition

The milk yield for the Jersey herd increased significantly ($p < 0.05$) by 5% during the Mootral period compared to before Mootral supplementation. The increased milk yield during Mootral supplementation was also recorded for the HF herd, which had a significant ($p < 0.05$) increase of 7.8% compared to before Mootral supplementation. Both herds' milk yield remained at the heightened level for 4 weeks after Mootral supplementation stopped (**Table 2**).

Milk constituents were monitored at pre-Mootral and during Mootral supplementation (**Table 3**) with most of the milk constituents (milk fat, protein,

Table 2. DMI and milk yield of Jersey and HF herds.

Item	Pre-Mootral		Mootral		Post-Mootral	
	Mean	SEM	Mean	SEM	Mean	SEM
Jersey herd (n = 121)						
DMI, kg/cow/day	13.25[a]	0.09	12.43[b]	0.21	11.34[c]	0.28
Milk yield, L/cow/day	22.33[a]	0.22	23.43[b]	0.14	23.55[b]	0.23
HF herd (n = 275)						
DMI, kg/cow/day	22.23[a]	0.12	23.44[a]	0.36	21.95[a]	0.63
Milk yield, L/cow/day	27.67[a]	0.34	29.83[b]	0.16	29.66[b]	0.16

Superscripts within the same row which differ denote significant differences ($p < 0.05$).

Table 3. Milk constituents of Jersey and HF herds before and during Mootral supplementation.

Item	Pre-Mootral		Mootral	
	Mean	SEM	Mean	SEM
Jersey herd (n = 121)				
Milk fat, %	5.64	0.04	5.64	0.02
Milk protein, %	4.12	0.03	4.09	0.01
Urea, mg/dL	8.33*	2.70	16.83*	3.42
pH	6.78	0.03	6.72	0.02
BTSCC[1]	84.16	7.43	82.30	6.89
HF herd (n = 275)				
Milk fat, %	4.18	0.02	4.34	0.02
Milk protein, %	3.46	0.02	3.43	0.01
Urea, mg/dL	7.67*	2.53	18.16*	0.89
pH	6.78	0.02	6.74	0.02
BTSCC[1]	140.16*	12.75	94.40*	5.07

[1]BTSCC = bulk tank somatic cell counts in milk, ×1000 cells/ml. Asterisks (*) denote statistically significant differences, $p < 0.01$.

and pH) analyzed in both herds showed no significant differences between periods, except for urea and BTSCC. This was unsurprising, as previous studies had not seen any negative effects of garlic on rumen fermentation [19] [20]. Although the urea content of the milk in both herds increased significantly during Mootral supplementation by 8.5 to 10.5 mg/dL ($p < 0.01$), the values were within the expected range (15 to 30 mg/dL) for cows in mid-lactation [21].

Only the BTSCC for the HF herd decreased significantly by 32.6% ($p < 0.01$) during the Mootral period. SCC reductions have been previously reported for a similar garlic-based supplement [22] [23]. In addition, numerous previous studies have suggested garlic and its sulphur constituents had an immune-modulating effect [24] [25] [26], which could have attributed to the lowering of SCC seen in this study. BTSCC is a quick gauge of the herd's health, whereby high numbers indicate an immune response to some likelihood of infection in the herd [27] [28]. The BTSCC for both herds are much lower than the European Commission's limit of ≤400,000 cells/ml [29] and milk processors often provide incentives to keep the BTSCC lower (≤250,000 cells/ml). If the effects of lower BTSCC from the Mootral supplement could be repeated in other dairy herds, including those with high SCC, it may prove economically attractive for farmers.

3.3. Feed Efficiency and Feed Analysis

This simple metric was used to gauge the herd's efficiency in converting feed into milk, which would eventually impact the farmer's net financial margin. The Jersey cows in this study showed a significant increase in FE during Mootral supplementation by 13% and increase of 24% after Mootral was withdrawn (**Figure 2(a)**), compared to the pre-Mootral period ($p < 0.05$). FE for the HF herd remained the same during Mootral supplementation compared to the pre-Mootral period, however increased by 8% ($p < 0.05$) during the post-Mootral period (**Figure 2(b)**). On average, the Jersey cows had a higher FE than the HF cows, which had been reported in a previous study [30]. Although the ruminal microbiome is responsible for the degradation of feed matter to provide energy for the cows, their population are not thought to be affected by the animals' genetics [31]. Though there was a difference in the FE trends observed during this study, it is not immediately understood and would warrant future investigation.

The significant reduction of enteric methane emission in the Jersey herd with Mootral supplementation might have contributed to the increase in their FE by 10% during the post-Mootral period. In the HF herd, the significant decrease of enteric methane emission did not have such a pronounced effect on their FE during Mootral supplementation, but the increase of their FE by 6% in the following 4 weeks after withdrawal of supplementation could be attributed to a persistent effect from the supplementation. Eger *et al.* [12] had shown that in the

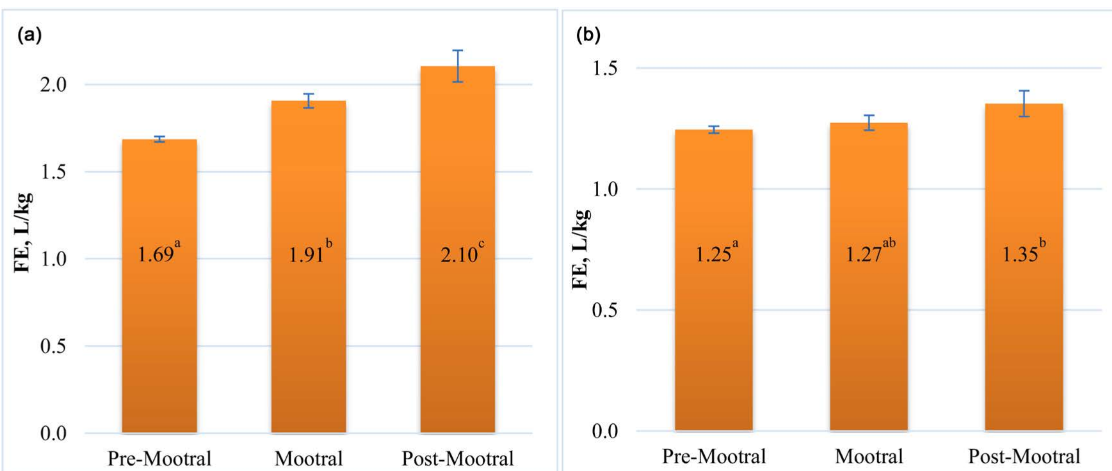

Figure 2. Feed efficiency (FE) for (a) Jersey herd (n = 121) and (b) HF herd (n = 275). FE = Milk yield/DMI (L/kg). Superscripts which differ indicate significant differences at $p < 0.05$. Error bars are SEM.

Table 4. Feed analysis of the total mixed rations fed to the Jersey and HF herds.

	Period		
Item	Pre-Mootral	Mootral	Post-Mootral
DM, g/kg	356	374	345
Composition, g/kg DM			
Crude ash	99	82	88
Crude protein	159	143	178
NDF	339	356	362
ADF	197	203	205
ME[1], MJ	10.2	10.4	10.4
NEl[2], MJ	6.1	6.3	6.2

[1]ME = Metabolized energy. [2]NEl = Net energy of lactation.

presence of Mootral, the percentage of methanogens reduced while bearing no negative effects on rumen fermentation. Our study here confirms these earlier findings especially with the lactating Jersey cows and to some extent in lactating HF cows with an improved FE in the longer term.

Results of feed analysis showed some changes in DM, crude ash, crude protein, NDF and ADF in the feed analysis but the changes were not statistically significant. The ME and NEL content were more consistent throughout the periods (Table 4).

There were no reports of adverse health events in both the Jersey and HF herds, and none of the cows had fertility, feet, mastitis, or metabolic issues during the study.

4. Conclusion

This was the first on-farm study of the Mootral supplement, using a commercial dairy farm, on the mitigation of enteric methane production in lactating cows and it confirmed the efficacy of the composition from prior *in vitro* work. The products of rumen fermentation is largely influenced by the diet of the cow [10] and these results may not be typical for all dairy farms, however the results encourages further exploration for the long term application of Mootral in ruminant livestock feeding to reduce enteric methane production. Although no negative impact on milk yield and quality was observed in this study, there was an indication of an immune effect. Future studies could encompass more parameters to evaluate other productivity effects and calculate the economic impact of an industry-wide application.

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Conflicts of Interest

H. Vrancken and M. Suenkel are employees of Mootral SA, which was the funding institute for the study. The rest of the authors declare no conflict of interest.

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